

REMARKS

Favorable reconsideration and allowance of the subject application are respectfully requested.

Applicants confirm the election to prosecute the invention of Group I, i.e. methods of cloning. Claims 10-32 have been replaced by claims 33-63.

Applicants also note that certified copies of the priority documents have not been received. Applicants will be submitting these documents as well as a declaration shortly.

The Invention

The inventors of the subject application have for the first time demonstrated the cloning of a genetically diverse repertoire of immunoglobulin variable region encoding sequences directly for expression of a library. Nobody previously suggested that one could or should do this. Important features of the present invention are:

- (i) It is a genetically diverse repertoire of sequences which is cloned.
- (ii) The sequences encode immunoglobulin variable domains, which are inherently variable.
- (iii) Back primers are used which will bind to a sequence at or adjacent the 3' end of the antisense strand of

target sequences containing variable domain coding sequences (i.e., to a repertoire of diverse sequences). The back primers prime the synthesis of the sense strand. This is very surprising given the inherent variability of these sequences, and has great utility and many advantages (discussed below).

- (iv) The cloning is into expression vectors for expression. Single domain ligands, Fv or Fab fragments expressed from these vectors can be selected by antigen binding.

As discussed on page 11 of the specification, prior to the subject invention, cloning of an immunoglobulin variable domain coding sequence required many steps. Individual probes were used in the isolation of a particular sequence of interest. This required sequencing of the N-terminal region of an antibody to enable design of the probes which would be used to isolate, and then perhaps amplify, a particular coding sequence in a cloning vector. It had not been contemplated previously that one should be able to design primers which would enable cloning of a repertoire of variable region genes, nor indeed that one could clone such a repertoire for expression.

The cloning of the entire vast repertoire of variable domain sequences which are found in, for example, the heterogeneous population of lymphocytes of a human would have

taken a very, very long time indeed if methods known before the present invention had been adopted. The sequencing of at least part of an individual antibody, design of suitable probes/primers for it, isolation of encoding nucleic acid, with possible amplification, followed by cloning into a cloning vector, perhaps with sequencing to confirm what has been obtained, eventual cloning into an expression vector, for each variable region to be cloned, would be a mammoth task. It would have been a task that the person skilled in the art would not have thought of attempting. Efforts to clone antibody sequences were aimed at monoclonal hybridomas, from which one can isolate a large amount of nucleic acid encoding an antibody of a particular specificity. Efforts in this area were directed to cloning in standard cloning vectors, allowing characterization of sequences obtained before re-cloning to expression vectors. The present inventors discovery of cloning a repertoire of immunoglobulin variable genes for expression is wholly new and provides basis for the present invention.

The present invention enables for the first time the rapid cloning for expression of a repertoire of variable domain sequences, whether derived from mRNA or DNA, cDNA or genomic DNA, from rearranged or unarranged genes. The methods rely on the existence of suitable primers for the target sequences. It is important to note that there are target sequences (plural) for the invention involves cloning a repertoire.

To clone target sequences, one needs oligonucleotide primers which will bind specifically at or adjacent each end of the target sequences. In the case of rearranged variable gene sequences one can find primers which will bind the sense strand at the 3' end of the variable domain coding region, for this is where the sequences lead into a JH coding segment known to be relatively conserved (see page 16 of the specification). Also, the target sequences encode the heavy chain fragment of a Fab fragment, then one can prime using a primer which will bind a sequence within the CH1 constant domain, again conserved. However, the present inventors have surprisingly found that the 5' ends of variable domain coding sequences are sufficiently conserved to permit design and use general back primers enabling cloning of a repertoire of variable domain coding sequences.

This finding enables specific copying of the antisense strands of the variable domain coding sequences into complementary sense strands. When used along with a forward primer which binds the sense strand at a sequence at the 3' end of the variable domain coding region, the general back primer enables cloning of target sequences containing only variable domain coding sequences with no extra nucleotides, if desired. If the forward primer binds the sense strand within the CH1 coding region then target sequences containing only Fab fragment coding sequences will be cloned.

The cloning may start from repertoire of mRNA isolated from a population of cells expressing antibodies. If a very large amount of mRNA is available it may not be necessary to amplify the cloned target sequences before insertion into expression vectors. The Examiner's objection that the claims would be directed to a method of amplification (discussed below) is therefore misfounded.

As already stressed, there is more to the methods of the invention than the primers. Because of their inherent properties, their sequences, the use of the primers in the methods affords great benefit. However, the invention includes the ideas of cloning a repertoire of variable domain coding sequences for expression. It provides for direct immortalization of an antibody repertoire of an animal, without the need to use complicated and expensive hybridoma techniques. Also, if the target sequences are derived from an animal immunized with a particular antigen, then the method of the invention can lead to a library of expression vectors from which a repertoire of antibodies or antibody fragments with binding specificity for that antigen can be expressed and selected. This selection may be directly by the binding specificity of the antibodies for the antigen of interest.

The present invention additionally provides for expression of cloned heavy chain variable regions in combination with

cloned light chain variable regions. The cloned variable regions can be expressed as fusion proteins with constant regions which have been inserted into the expression vectors before the variable regions, or as fusions with, for example, peptide tags or bacterial or phage surface proteins.

Where a combinatorial approach is adopted, combining a repertoire of heavy chains with a repertoire of light chains, a vast library pool of possible combinations can be achieved. Using binding affinity for an antigen, antibodies with the desired specificity can be selected from the vast pool, bypassing conventional monoclonal antibody technology.

The Art Rejections

The art cited in the subject Official Action has been carefully considered by the Applicants together with the examiner's comments relative thereto and, in response, the claims have been rewritten in an effort to more particularly point out and distinctly claim the subject invention.

None of the documents relied on by the Examiner - Mullis, Skerra, Herzog, Kabat and Schoemaker - make any suggestion of the methods of the invention as presently claimed. None makes any suggestion that one could or should clone repertoires of immunoglobulin variable region encoding sequences, nor that one should do this for expression without the need for intervening

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sequencing and/or re-cloning. No-one previously cloned variable domain repertoires for expression.

Claims 10, 11, 14-18, and 27 stand rejected under 35 U.S.C. §102(b) as being anticipated by Mullis et al (A). Reconsideration is requested.

Mullis et al disclosed PCR generally with some broadening statements. The idea of the present invention and its realization is not envisaged by the disclosure of Mullis et al. There is no disclosure or suggestion in Mullis that one could or should clone repertoires of immunoglobulin variable domain coding sequences for expression. The fourth full paragraph of column 2 of Mullis is a brief description of some features of that invention but it provides no motivation to arrive at the present invention. There is no mention of variable domains and the problems associated with cloning a repertoire of encoding sequences for expression.

Column 8, the passage referred to by the Examiner, deals with a situation where part of a protein has been sequenced, but because of the degeneracy of the genetic code one cannot say what the correct encoding nucleic acid sequence is. In such a situation, Mullis et al suggest using a mixture of primers. This says nothing about whether one could or should adopt the methods of the present invention to clone a repertoire of

immunoglobulin variable domain encoding sequences for expression. In the present situation, the nucleic acid sequences for the variable domains are inherently variable, a situation totally different from that envisaged by Mullis et al. They are suggesting how to obtain one nucleic acid sequence of interest when only the amino acid sequence is known. The present invention does away with the need to do any amino acid sequencing. It was previously unknown that one could use back primers for cloning a repertoire of inherently variable immunoglobulin variable domain coding sequences for expression. Mullis provides no teaching on this.

In cloning immunoglobulin variable domain sequences, an analogous approach to that of Mullis would be to sequence the end of each sequence to be cloned and then design and make primers specific for each one. Such an approach is avoided by the present invention and would not be feasible for a repertoire of millions of sequences. As already discussed, the present invention breaks away from the previously standard cloning techniques and is a bold technological advance.

Claims 10, 11, 14-19, 22, 27, 29, 30, and 32 stand rejected under 35 U.S.C. §103 as being unpatentable over Mullis et al (A). Reconsideration is requested.

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The Examiner's attention is redirected to the Applicant's response to the above art rejection.

Claims 1-22 and 26-32 stand rejected under 35 U.S.C. §103 as being unpatentable over Skerra et al (R) in view of either Mullis et al (A) or Herzog et al (B), and in view of Kabat et al (S). Reconsideration is requested.

Skerra et al teach no more than the cloning and expression in E. coli of DNA segments encoding Fv fragments of a single specific antibody (for the antigen McPC603). The amino acid sequence of the antibody was known and the workers synthesized the genes for both the VL and VH domains which they then expressed. (See page 1039, in particular the third column). There is absolutely no teaching or suggestion of the present invention, involving cloning repertoires of many different variable domain sequences for expression.

Herzog et al are largely concerned with probes for detection of DNA, and specifically only concerned with probing for human papilloma virus nucleic acid. The document is unconcerned with immunoglobulin variable domains and provides no motivation to the person skilled in the art to clone repertoires of immunoglobulin variable domain encoding sequences for expression and certainly no teaching as to how to do this. Explicitly, in Example 6, Herzog et al describe that the

sequences of the primers were "selected so as to amplify specifically the fragment of the gene of the virus to which they were addressed, with the exclusion of all the others" (emphasis added). The primers were designed to bind part of one virus and that virus only. Once again, this is totally different from the present invention which relied on primers which will enable cloning of a repertoire by binding many variable domain coding sequences. Arguably, were Herzog et al to be considered at all relevant to the present invention (even though it is unconcerned with variable domain sequences and cloning of a repertoire for expression), then it would be teaching away.

Kabat et al gives no indication that it is possible to clone for expression a repertoire of variable domain coding sequences.

The Examiner has asserted that "It would have been obvious to one of ordinary skill in the art to have modified the teachings of Skerra et al by using mixed primer PCR, as taught by either Mullis et al or Herzog et al, based upon the DNA sequences taught by Kabat et al." It is respectfully submitted that the documents, firstly, provide no grounds for their combination in this way, and, secondly, do not in any event afford a conclusion of obviousness of the present invention. Clearly, the present invention is not merely mixed primer PCR.

Of Mullis, Skerra and Herzog, only Skerra is at all concerned with immunoglobulin. In fact Skerra is only concerned with one immunoglobulin Fv fragment of known amino acid sequence. As mentioned above, Skerra et al synthesized the genes they expressed using their knowledge of the amino acid sequence. This has absolutely nothing to do with cloning of a repertoire of many inherently viable sequences. To operate the methods of the present invention one need not know the sequences of any of the variable domains being cloned. Skerra is irrelevant to the present invention as claimed. There is nothing in the document to motivate a combination of its teaching with Mullis or Herzog, and its teaching is irrelevant.

Neither Mullis nor Herzog teach or suggest the problems solved by the present invention, for they are simply not concerned with cloning repertoires of immunoglobulin variable domain coding sequences. The person skilled in the art, concerned with cloning antibody variable regions would have no reason to turn to Herzog et al.

Likewise, as discussed above, Mullis et al neither suggest nor address the problems associated with cloning a repertoire of inherently variable coding sequences for immunoglobulin variable domains. Combining Mullis et al with any of the other documents does not alter this.

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Claims 23-25 stand rejected under 35 U.S.C. §103 as being unpatentable over Skerra et al in view of either Mullis et al or Herzog et al, and in view of Kabat et al as applied to claims 1-22 and 26-32 above, and further in view of Schoemaker et al (C). Reconsideration is requested.

Skerra, Mullis, Herzog and Kabat are discussed above. Schoemaker et al disclose so-called "heterochain antibodies" consisting of heavy and light chains from different sources. They do not envisage cloning of repertoires by the claimed method, nor do they suggest producing a combinatorial library of heavy and light chains of many different specificities. Rather, they concern themselves with taking a heavy chain from one antibody of known specificity and combining it with a light chain from a different antibody of the same specificity, to produce a heterochain antibody which might be better than the "parents."

Applicants respectfully submit that none of the cited art of record, either taken alone or in combination, discloses, suggests or renders obvious the invention as claimed herein.

35 U.S.C. §112 Rejections

Claims 27-30 stand rejected under 35 U.S.C. §112, first and second paragraphs. Reconsideration is requested.

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Claims 27-30 have been cancelled, obviating the need to comment on this rejection.

Claims 1-32 stand rejected under 35 U.S.C. §112, first paragraph. Reconsideration is requested.

The Examiner has objected that the disclosure is enabling only for claims limited to the PCR primers disclosed and therefore to the mammalian immunoglobulin genes amplified using said primers.

The invention is much more than the provision of specific primers. The invention relies on fundamental ideas which were not previously suggested, i.e., the cloning of a repertoire, the repertoire consisting of variable region sequences, and the cloning being into expression vectors for expression. The contribution which the present inventors have made to the art includes not only the knowledge that it is possible to clone repertoires of variable region sequences for expression, highly inventive in itself, but also the complete methods of doing this. They have also provided means for putting the methods into effect and have demonstrated success with a variety of genes from various sources. The skilled person could easily design primers other than those specifically disclosed and practice the method of the invention. The invention is much more than the disclosed primers, and without the benefit of the

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present invention, the person skilled in the art would not have had any inkling that he could or should adopt the approaches claimed. The Applicants are entitled to claims with scope commensurate with the valuable contribution the present invention has made to the art.

Claims 10-13, 15, 16, 21, 22, 24, 25, 27-29, and 32 stand rejected under 35 U.S.C. §112, second paragraph.  
Reconsideration is requested.

The methods are methods of cloning, as discussed above. Amplification of cloned target sequences might be desirable in some instances but is not essential for the invention.

"Hybridise" now reads "hybridize."

Step (a), of providing nucleic acid, is important because the double-stranded nucleic acid may be provided from any one of a number of different sources (as discussed). The double-stranded nucleic acid can be provided as RNA/DNA heteroduplexes formed by reverse transcription, as exemplified. Additionally, claims 4 and 5 are directed to the provision of double-stranded nucleic acid starting from single-stranded nucleic acid, such as mRNA.

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"Plurality of times" has a clear meaning. It is apparent that there is no upper limit to the number of cycles which can be performed when amplifying target sequences. It depends on how much nucleic acid one starts with and how much one wants to end up with. The person skilled in the art would understand this.

The non-statutory wording of previous claims 12-13 does not now appear.

"Closely-related" has clear meaning in the context of the description read as a whole.

The same applies to "species specific general." A primer described thus is one which is useful for cloning any desired immunoglobulin variable domain encoding sequences from a specific species - see page 27, first complete paragraph.

"Sequence which is annealed" no longer appears.

"Expressed alone" means expressed without anything else. This is clear in isolation and in context.

Previous claims 24, 25, 28, 29 and 32 no longer appear. "One or more" need not specify an upper limit because there is

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no upper limit. Other issues raised by the Examiner have been addressed in the method claims presented herein.

Conclusions

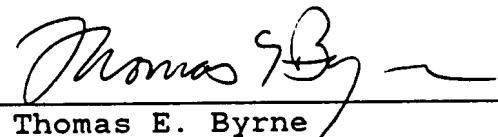
In view of the above, Applicants respectfully submit that all claims now pending herein fully and patentably define the present invention over the applied art of record. As such, early receipt of the official Notice of Allowance is awaited.

Should any small matters remain outstanding, the Examiner is encouraged to telephone Applicants' undersigned attorney so that same can be resolved without the necessity of an additional action and response thereto.

Respectfully submitted,

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